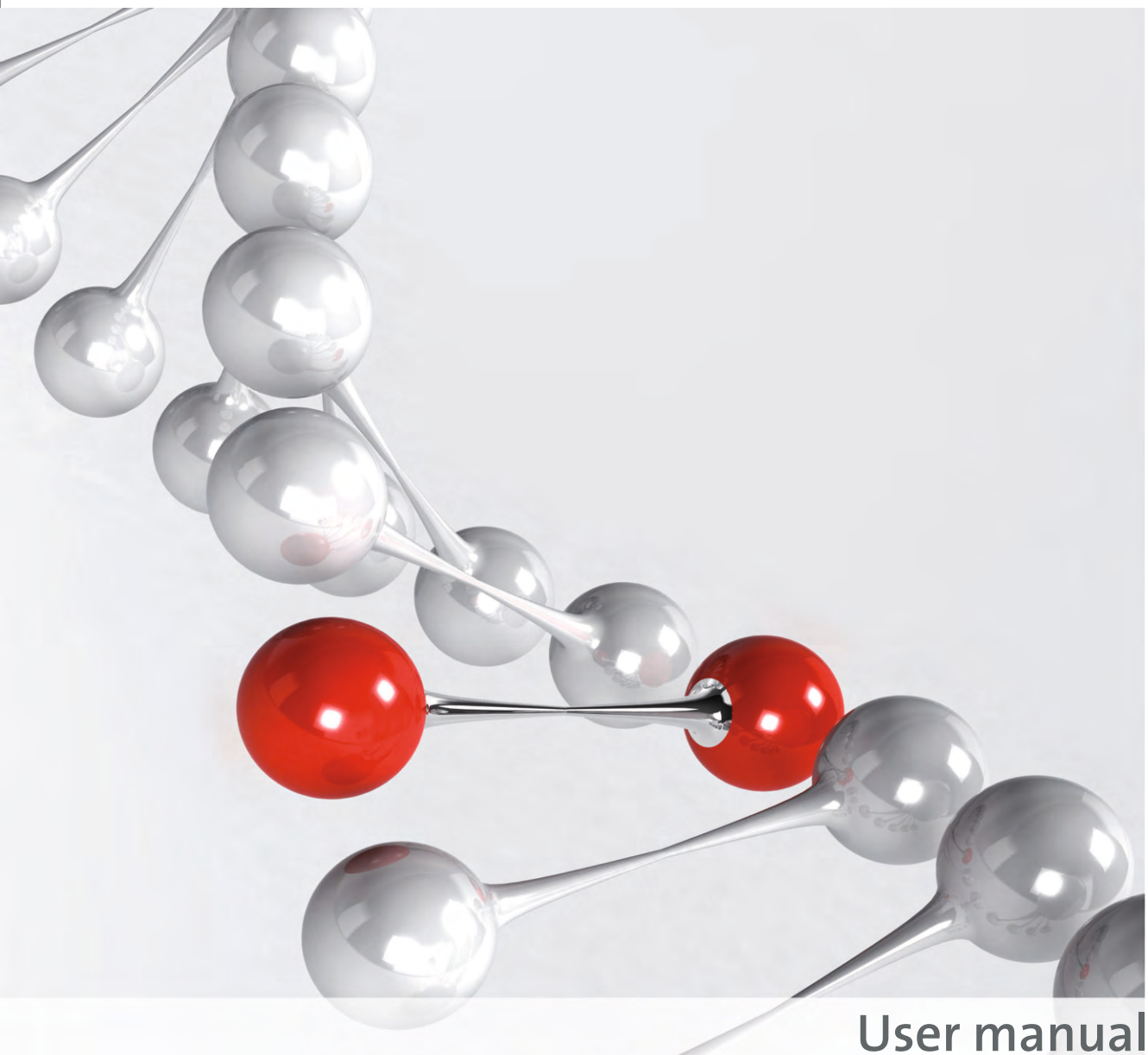
stratec
molecular



User manual
InviMag® Universal Kit/ KF96

for use on KingFisher® 96 and KingFisher® Flex, Thermo Fisher Scientific

for automated purification of DNA (genomic, bacterial, mitochondrial and viral) as well as viral RNA from up to 200 µl of clinical samples with magnetic beads

REF 7450300X0 STRATEC Molecular GmbH, D-13125 Berlin

Instruction for InviMag® Universal Kit/ KF96

The **InviMag® Universal Kit/ KF96** combines the advantages of the innovative InviMag® technology with easy handling of magnetic particles in combination with either the KF96 or KFflex96 robotic platform from Thermo Fisher Scientific for a very efficient and reliable isolation of nucleic acids with a high purity. The kit is the ideal tool for semi-automated isolation and purification of total (genomic, bacterial) DNA and/or viral DNA/RNA from up to 200 µl sample volume.

The interplay of the nucleic acid extraction and purification chemistry provided by the **InviMag® Universal Kit/ KF96** was intensely tested and validated.

The nucleic acid binding particles are characterized by a high surface area, uniform size distribution, good suspension stability and therefore are highly suitable for high-throughput processing.

Due to the high purity of the derived eluates, the isolated nucleic acids are ready-to-use in a broad spectrum of downstream applications or can alternatively be stored at -20°C/-80°C for subsequent use.

For research use only!

Trademarks: InviMag®, Invisorb®. Registered marks, trademarks, etc. used in this document, even when not specifically marked as such, are not to be considered unprotected by law.

The Invisorb® technology is covered by patents and patent applications: US 6,110363, US 6,043,354, US 6,037,465, EP 0880535, WO 9728171, WO 9534569, EP 0765335, DE 19506887, DE 10041825.2, WO 0034463.

InviMag® and Invisorb® are registered trademarks of STRATEC Biomedical AG.

The PCR process is covered by US Patents 4,683,195, and 4,683,202 and foreign equivalents owned by Hoffmann-La Roche AG.

© 2015 STRATEC Molecular, all rights reserved.

Table of content

Kit contents of InviMag® Universal Kit/ KF96 **3**
Kit contents of InviMag® Universal Kit/ KF96 w/o plastic **4**
Symbols **5**
Storage **5**
Quality Control and product warranty **5**
Intended use **6**
Product use limitation **6**
Safety information **7**
Product characteristics of the InviMag® Universal Kit/ KF96 **8**
Sampling and storage of starting material **9**
Principle and procedure **10**
Procedure **10**
Yield and quality of genomic DNA derived from blood **10**
Yield and quality of viral nucleic acids **11**
Protocol validation **11**
Important notes **14**
Preparing reagents and buffers **14**
Reagents and equipment to be supplied by user **15**
Important indications **15**
Scheme of the InviMag® Universal Kit/ KF96 **16**
Lysis procedures **17**
Protocol 1: Simultaneous isolation of nucleic acids (viral DNA/RNA) from cell-free body fluids or blood (genomic DNA) 17
Protocol 2: Simultaneous isolation of nucleic acids (DNA/RNA) from swab material 18
Protocol 3: Simultaneous isolation of nucleic acids (DNA and RNA) from tissue biopsies 19
Protocol 4: Isolation of DNA from bacteria pellets (up to 1 x 10^9 bacterial cells) 19
Protocol 5: Simultaneous isolation of total nucleic acids from sputum 20
Protocol 6: Simultaneous isolation of total nucleic acids (DNA and RNA) from tracheal secretes or BAL 20
Protocol 7: Simultaneous isolation of viral nucleic acids from stool samples 22
Protocol 8: Isolation of bacterial DNA from stool samples 22
Starting a Run **23**
For self-programming of the KF96 and KFflex96 instrument **25**
Troubleshooting **28**
Appendix **30**
General notes on handling DNA **31**
General notes on handling RNA **32**
Ordering information **33**

Kit contents of InviMag® Universal Kit/ KF96

Store the **MAP Solution B** and **Carrier RNA** at 2-8°C! Store dissolved **Proteinase K / Carrier RNA** in aliquots at -20°C! Store all other kit components at room temperature (RT*)!

Component	1 x 96 preparations	5 x 96 preparations
Catalogue No.	7450300100	7450300200
Lysozyme Buffer	15 ml	15 ml
Lysozyme	150 mg	150 mg
Lysis Buffer HLT	30 ml	120 ml
Carrier RNA	2 x 1.2 ml working solution	15 ml working solution
RNAse Free Water	3 x 2 ml	2 x 15 ml
Proteinase K	2 x 1.1 ml working solution	10.5 ml working solution
SNAP Solution	2 x 1.1 ml	10.5 ml
Binding Solution (fill with 99.7% Isopropanol)	empty bottle (final volume 30 ml)	empty bottle (final volume 120 ml)
Wash Buffer HLT	90 ml (final volume 150 ml)	360 ml (final volume 600 ml)
Wash Buffer II	45 ml (final volume 150 ml)	180 ml (final volume 600 ml)
Wash Buffer M	30 ml (final volume 120 ml)	150 ml (final volume 600 ml)
Elution Buffer M	15 ml	60 ml
KF96 Tip Comb for DW magnets	1	5
2.0 ml Deep Well Plate	4	5 x 4
200 µl Elution Plate	2	5 x 2
Sealing Foils	2	10
1.5 ml Receiver Tubes	2 x 50 pcs.	10 x 50 pcs.
Manual	1	1
Initial steps	Add 60 ml of abs. 99.7% Isopropanol to the bottle **Wash Buffer HLT**, mix thoroughly and keep the bottle firmly closed! Add the provided amount of **Lysozyme** to the bottle with the **Lysozyme Buffer** and mix it thoroughly Add 90 ml of 96-100% ethanol to the bottle **Wash Buffer M**, mix thoroughly and keep the bottle firmly closed! Add 105 ml of 96-100% ethanol to the bottle **Wash Buffer II**, mix thoroughly and keep the bottle firmly closed! Resuspend each tube **Carrier-RNA** in 1.2 ml RNAse free water. Mix thoroughly until completely dissolving. Resuspend each tube **Proteinase K** in 1.1 ml RNAse free water, mix thoroughly until completely dissolving and store at -20°C! Fill 30 ml 99.7% **Isopropanol** (molecular biologic grade) into the empty bottle.	Add 240 ml of abs. 99.7% Isopropanol to the bottle **Wash Buffer HLT**, mix thoroughly and keep the bottle firmly closed! Add the provided amount of **Lysozyme** to the bottle with the **Lysozyme Buffer** and mix it thoroughly Add 450 ml of 96–100% ethanol to the bottle **Wash Buffer M**, mix thoroughly and keep the bottle firmly closed! Add 420 ml of 96-100% ethanol to the bottle **Wash Buffer II**, mix thoroughly and keep the bottle firmly closed! Resuspend the **Carrier-RNA** in 15 ml RNAse free water. Mix thoroughly until completely dissolving. Resuspend the **Proteinase K** in 10.5 ml RNAse free water, mix thoroughly until completely dissolving and store at -20°C! Fill 120 ml **99.7% Isopropanol** (molecular biologic grade) into the empty bottle.

Kit contents of InviMag® Universal Kit/ KF96 w/o plastic

Store the **MAP Solution B** and **Carrier RNA** at 2-8°C! Store **dissolved Proteinase K / Carrier RNA** in aliquots at -20°C! Store all other kit components at room temperature (RT*)!

Component	1 x 96 preparations	5 x 96 preparations
Catalogue No.	7450300150	7450300250
Lysozyme Buffer	15 ml	15 ml
Lysozyme	150 mg	150 mg
Lysis Buffer HLT	30 ml	120 ml
Carrier RNA	2 x 1.2 ml working solution	15 ml working solution
RNAse Free Water	3 x 2 ml	2 x 15 ml
Proteinase K	2 x 1.1 ml working solution	10.5 ml working solution
SNAP Solution	2 x 1.1 ml	10.5 ml
Binding Solution (fill with 99.7% Isopropanol)	empty bottle (final volume 30 ml)	empty bottle (final volume 120 ml)
Wash Buffer HLT	90 ml (final volume 150 ml)	360 ml (final volume 600 ml)
Wash Buffer II	45 ml (final volume 150 ml)	180 ml (final volume 600 ml)
Wash Buffer M	30 ml (final volume 120 ml)	150 ml (final volume 600 ml)
Elution Buffer M	15 ml	60 ml
Sealing Foils	2	10
1.5 ml Receiver Tubes	2 x 50 pcs.	10 x 50 pcs.
Manual	1	1
Initial steps	Add 60 ml of abs. 99.7% Isopropanol to the bottle **Wash Buffer HLT**, mix thoroughly and keep the bottle firmly closed! Add the provided amount of **Lysozyme** to the bottle with the **Lysozyme Buffer** and mix it thoroughly Add 90 ml of 96-100% ethanol to the bottle **Wash Buffer M**, mix thoroughly and keep the bottle firmly closed! Add 105 ml of 96-100% ethanol to the bottle **Wash Buffer II**, mix thoroughly and keep the bottle firmly closed! Resuspend each tube **Carrier-RNA** in 1.2 ml RNAse free water. Mix thoroughly until completely dissolving. Resuspend each tube **Proteinase K** in 1.1 ml RNAse free water, mix thoroughly until completely dissolving and store at -20°C! Fill 30 ml 99.7% **Isopropanol** (molecular biologic grade) into the empty bottle.	Add 240 ml of abs. 99.7% Isopropanol to the bottle **Wash Buffer HLT**, mix thoroughly and keep the bottle firmly closed! Add the provided amount of **Lysozyme** to the bottle with the **Lysozyme Buffer** and mix it thoroughly Add 450 ml of 96–100% ethanol to the bottle **Wash Buffer M**, mix thoroughly and keep the bottle firmly closed! Add 420 ml of 96-100% ethanol to the bottle **Wash Buffer II**, mix thoroughly and keep the bottle firmly closed! Resuspend the **Carrier-RNA** in 15 ml RNAse free water. Mix thoroughly until completely dissolving. Resuspend the **Proteinase K** in 10.5 ml RNAse free water, mix thoroughly until completely dissolving and store at -20°C! Fill 120 ml **99.7% Isopropanol** (molecular biologic grade) into the empty bottle.

Plastic to be supplied	(see order information)	
2.0 ml Deep Well Plate	4 pcs.	5 x 4 pcs.
KF96 Tip Comb for DW	1 pcs.	5 pcs.
200 µl Elution Plate	2 pcs.	5 x 2 pcs.

Symbols

Manufacturer

Lot number

Catalogue number

Expiry date

Consult operating instructions

Temperature limitation

Do not reuse

Storage

All buffers and kit contents of the **InviMag® Universal Kit/ KF96**, except **Carrier RNA**, **dissolved Proteinase K** and **SNAP Solution,** should be stored at room temperature and are stable for at least 12 months at this condition.

Carrier RNA/ Proteinase K: Lyophilized **Carrier RNA** should be stored at 2-8°C. Dissolved **Carrier RNA** and **Proteinase K** must be stored at -20°C in aliquots.

SNAP Solution: The magnetic beads should be stored at 2-8°C.

Wash Buffer M/ Wash Buffer II / Wash Buffer HLT: Wash Buffers, charged with either ethanol or isopropanol should be stored at room temperature and must be appropriately sealed. If any precipitates are visible within the provided solutions, solve them by carefully warming up to 30°C.

Room temperature (RT) is defined as range from 15-30°C.

Quality Control and product warranty

STRATEC Molecular guarantees the correct function of the **InviMag® Universal Kit/ KF96** for applications as described in the manual. In accordance with STRATEC Molecular's certified QM-System each component of the **InviMag® Universal Kit/ KF96** was tested against predetermined specifications to ensure consistent product quality.

All products sold by STRATEC Molecular are subjected to extensive quality control procedures according to ISO 9001:2008 and ISO 13485:2003+AC:2009 and are warranted to perform as described when used correctly. Any problems should be reported immediately.
STRATEC Molecular reserves the right to change, alter or modify any product to enhance its performance and design at any time.

If you have any questions or problems regarding any aspects of **InviMag® Universal Kit/ KF96** or other STRATEC Molecular products, please do not hesitate to contact us**.**

For technical support or further information please contact:
from Germany: +49-(0)30-9489-2901/ 2910
from abroad: +49-(0)30-9489-2907/2903 or contact your local distributor.

Intended use

The **InviMag® Universal Kit/ KF96** is designed for semi-automated rapid and economical preparation of nucleic acids (viral DNA/RNA, genomic DNA, bacterial DNA but not plasmid DNA) from 200 µl sample volumes like blood (EDTA/Citrate stabilized, but not heparin), serum, plasma, cerebrospinal fluid, cell culture supernatant, cell-free body fluids, urine, supernatant from stool suspensions, rinse liquid from swabs or bacterial suspensions, sputum, BAL using magnetic beads and the KF96 / KFflex96 instrument from Thermo Fisher Scientific.

The whole process is based on the patented **InviMag®** technology, which relies on binding of the nucleic acids by magnetic particles. The procedure only requires minimal user interaction (prefilling of the plates), allowing safe handling of potentially infectious samples.

The isolation protocols and buffers are optimized to provide high yields and purities. However, for reproducible yields appropriate sample storage and quick handling is essential. The purified viral DNA and/or RNA as well as bacterial or genomic DNA are ready-to-use for downstream analysis.

THE PRODUCT IS INTENDED FOR USE BY PROFESSIONALS, SUCH AS TECHNICIANS, PHYSICIANS AND BIOLOGISTS TRAINED IN MOLECULAR BIOLOGICAL TECHNIQUES.

The generated eluates can be used with any downstream application, employing enzymatic amplification or other modifications of DNA/RNA followed by signal detection or amplification. Any diagnostic results generated, using the sample preparation procedure in conjunction with any downstream diagnostic assay, should be interpreted with regards to other clinical or laboratory findings.

All utilities, except Ethanol and Isopropanol, required for preparation of nucleic acids are provided by the **InviMag® Universal Kit/ KF96.**

To minimize irregularities in diagnostic results, adequate controls for downstream applications should be used.

Product use limitation

The kit is validated for e.g. viral DNA/RNA extraction from cell-free body fluids and rinsed liquids, specifically for human serum and plasma. Related applications will need a separate validation. Extraction of other than human DNA from blood or of total RNA has not been evaluated with this kit. The included chemicals are only useable once.

Differing the starting material or flow trace may lead to inoperability. Therefore neither a warranty nor a guarantee in this case will be given, implied or expressed.

The user is responsible to validate the performance of the STRATEC Molecular product for any particular use. STRATEC Molecular does not provide validations of performance characteristics of the product with respect to specific applications. STRATEC Molecular products may be used in clinical diagnostic laboratory systems conditioned upon the complete diagnostic system of the laboratory. The laboratory must be validated pursuant to CLIA' 88 regulations in the U.S. or equivalents in other countries.

All products sold by STRATEC Molecular are subject to extensive quality control procedures (according to ISO 9001-2000 and ISO EN 13485) and are warranted to perform as described herein. Any problems, incidents or defects shall be reported to STRATEC Molecular immediately upon detection thereof.

The chemicals and the plastic parts are for laboratory use only. They must be stored in the laboratory and must not be used for other purposes than intended.

The included chemicals are only useable once and are not suitable for consumption.

Safety information

When and while working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles!

Avoid skin contact with reagents and samples! Adhere to the legal requirements for working with biological material!

For more information, please consult the appropriate material safety data sheets (MSDS). These are available online in convenient and compact PDF format at **www.stratec.com** for each STRATEC Molecular product and its components. If buffer bottles are damaged or leaking, WEAR GLOVES, AND PROTECTIVE GOGGLES when discarding the bottles in order to avoid any injuries.

STRATEC Molecular has not tested the liquid waste generated by the **InviMag® Universal Kit/ KF96** procedures for residual infectious materials. Contamination of the liquid waste with residual infectious materials is highly unlikely, but cannot be excluded completely. Therefore, liquid waste must be considered and handled as infectious and discarded accordingly to local safety regulations.

European Community risk and safety phrases for the components of the **InviMag® Universal Kit/ KF96** to which they apply are listed below as follows:

Lysis Buffer HLT

contains guanidine-hydrochloride

warning

H302-315-319, P280-505+351+338

Proteinase K

danger

H315-319-334-335 P280-305-351-338-310-405

Wash Buffer HLT

contains guanidine-hydrochloride

warning

H302-315-319, P280-505+351+338

H302	Harmful if swallowed.
H315:	Causes skin irritation.
H319:	Causes serious eye irritation.
H334:	May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H335:	May cause respiratory irritation.
P280:	Wear protective gloves/protective clothing/eye protection/face protection.
P305+P351+P338:	If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses and continue rinsing.
P310:	Immediately call a POISON CENTER or doctor/physician.
P405:	Store locked up.

Emergency medical information can be obtained 24 hours a day from infotrac:

outside of USA: 1 – 352 – 323 – 3500
inside of USA: 1 – 800 – 535 – 5053

Product characteristics of the InviMag® Universal Kit/ KF96

Starting Material	Yield	Time for preparation
up to 200 µl cell-free body fluids like serum, plasma or liquor etc. up to 200 µl rinsed liquid from swabs up to 200 µl cell culture supernatants up to 200 µl blood (EDTA / Citrate stabilized, but not heparin) 1x 10^6 mammalian cells 10 mg tissue sample	Depends on sample (storage and source) ***Note:*** The added Carrier RNA will account for most of the eluted nucleic acid(s). Quantitative (RT)-PCR is recommended for determination of the viral RNA or DNA yield	about 60 min

The **InviMag® Universal Kit/ KF96** is the ideal tool for an efficient and semi-automated extraction of genomic and/or bacterial DNA and viral DNA/RNA from different sample sources.

The nucleic acid isolation process is based on the interaction of nucleic acids with silica coated magnetic particles in presence of adjusted buffer conditions.

The DNA/ RNA purification procedure is performed with minimal user intervention, except the initial loading of the system and plate preparation. This allows safe handling of potentially infectious samples. Sample cross-contamination and reagent cross-over is effectively eliminated.

The King Fisher instrument uses magnetic rods to transport the DNA/RNA-binding magnetic particles through the various extraction phases: lysis, binding, washing and elution. The automated purification process results in a fast, reliable and robust technique.

After a sample specific lysis, using **Lysis Buffer HLT** and **Proteinase K** (and **Lysozyme** if required), optimal binding conditions are adjusted upon addition of **Binding Solution.** The genomic DNA and/or viral DNA/RNA will bind to the added magnetic particles (**SNAP Solution**) and is separated from the solution by magnetic rods controlled by the KingFisher system. Subsequent to three washing steps of the particle bound nucleic acids, the nucleic acids are finally eluted in **Elution Buffer M**.

Due to the high purity, the eluted nucleic acids are ready-to-use in a broad panel of downstream applications like:

- PCR*, real-time PCR
- Restriction Enzyme Digestion
- HLA Typing
- Southern Blot

For the isolation of DNA from single blood samples, STRATEC Molecular offers the Invisorb® Spin Blood Mini Kit or for 8–96 samples, the Invisorb® Blood Mini 96 HTS Kits for use on a centrifuge, vacuum manifold or robotic station.

For the isolation of viral RNA, DNA or both, STRATEC Molecular offers a series of spin kits as well as HTS kits for use on a centrifuge, vacuum manifold, or on other robotic stations as well as magnetic bead based kits (see page 23).

*) The PCR process is covered by US Patents 4,683,195, and 4,683,202 and foreign equivalents owned by Hoffmann-La Roche AG.

Sampling and storage of starting material

For reproducible and high yields the correct sample storage is essential. Yields may vary from sample to sample depending on factors such as health of the donor, sample age, kind of sample, transport and storage conditions.

Cultivated bacteria or bacterial suspension(s):
Bacteria have to be pelleted after cultivation and resuspended in defined conditions. Best results are obtained with fresh material.

Swabs, Saliva: The protocol works with fresh saliva, prepared swabs as well as with dried swabs. The protocol is not validated for the isolation of DNA from swabs provided in storage buffers from other providers.
Best results are obtained using freshly extracted samples. As long as the samples are not shock frosted in liquid nitrogen or incubated with RNase inhibitors or denaturing reagents, the viral RNA is not secured. Therefore, it is essential that samples are immediately flash frozen subsequent to harvesting by using liquid nitrogen and storage at -80°C. Viral RNA in deep frozen samples is stable for months. However, viral RNA purification should be processed as soon as possible.

Urine: The bacteria should be pelleted while the supernatant is discarded (urea contaminations can inhibit PCR reactions).For some applications fresh urine can be used directly. Best results are obtained with freshly pelleted material

Blood: Best results are obtained using fresh blood samples. Blood samples (stabilized with EDTA or Citrate but **not** heparin) can be stored at room temperature (18-30°C) for 2-3 hours. For short-term storage (up to 24 h) samples should be stored at 4-8°C. For long-term storage, we recommend to freeze the samples at -20°C or -80°C. Avoid multiple thawing and freezing cycles of the sample(s) before isolating the DNA/RNA because this may lead to degraded DNA.

Stool samples: Best results are obtained with fresh material. Stool samples contain DNases and RNases which quickly realize DNA and RNA digestion and degradation. The sample may be stored frozen at – 80°C.

Serum and plasma (and other cell-free body fluids): After collection and centrifugation, serum or plasma derived from blood (treated with anticoagulants like EDTA or Citrate, but not with heparin), synovial fluid samples or other cell-free body fluids and rinse liquids from swabs can be used for extraction. For short-term storage samples can be kept on ice for 1-2 hours. For up to 24 h samples may be stored at -20°C. For long-term storage, we recommend freezing samples in aliquots at –80°C. Frozen plasma or serum samples must not be thawed more than once. Multiple thawing and freezing cycles before isolating the viral DNA/RNA should be avoided because this may lead to denaturation/precipitation of proteins, resulting in reduced viral titers and therefore reduced yields. In addition, cryoprecipitates formed during freeze-thawing cycles can cause problems. If cryoprecipitate are visible, centrifuge them down at 6.800 x *g* for 3 minutes. The cleared supernatant should be aspirated, without disturbing the pellet and processed immediately. This step will not reduce viral titers.

Cell culture supernatants: Best results are obtained with fresh material or material that has been immediately frozen and stored at –20°C or –80°C after winning of the cell culture supernatant. Repeated freezing and thawing of stored samples can negatively influence the sensitivity.

STRATEC Molecular will be released of its responsibilities if other sample materials than described in the Intended Use are processed or if the sample preparation protocols are changed or modified.

Principle and procedure

The **InviMag® Universal Kit/ KF96** procedure comprises following steps:

- Sample preparation (if required)
- Lysis step
- Adjustment of binding conditions
- Binding of the nucleic acids to magnetic beads
- Washing of the bead bound nucleic acids and evaporation of ethanol
- Elution of nucleic acids

Procedure

Bacteria must be cultivated at special conditions. An aliquot of the bacteria suspension is used to achieve a bacterial pellet by centrifugation at high speed for 5 min while the supernatant is discarded.

Pretreatment: Please check the specific protocol section

Lysis
Samples are lysed at elevated temperatures in the presence of **Lysis Buffer HLT** and **Proteinase K** (**Lysozyme if required)** to break bacterial and viral cell walls and to digest proteins.

The addition of **Carrier RNA** is required for the enhancement and stabilization of viral DNA/RNA recovery. Due to this, it is even possible to purify very small amounts of viral DNA/RNA molecules.

If required add the extraction controls

Binding of the nucleic acids
After addition of **Binding Solution** to adjust optimal binding conditions, the nucleic acids are bound by the simultaneously added magnetic beads (**SNAP Solution**).

Removing residual contaminants
Contaminants are efficiently removed during the washing process using **Wash Buffer HLT**, **Wash Buffer M** and **Wash Buffer II** while the nucleic acids remain bound to the magnetic beads.

Elution
The nucleic acids are finally eluted in **Elution Buffer M** and are ready-to-use in different subsequent downstream applications, e.g. for PCR amplification, digestion with restriction enzymes, Southern hybridizations, HLA typing, etc.

Yield and quality of genomic DNA derived from Blood

The amount of purified DNA/RNA in the **InviMag® Universal Kit/ KF96** procedure from whole blood depends on the leucocytes content, the sample source, transport, storage and age.

Typically, a volume of 200 µl whole blood from a healthy individual with an elevated white blood cell content - ranging from 3 x 10^6 to 1 x 10^7 cells/ml - will yield 3 - 6 µg of genomic DNA. If a whole blood sample is mixed with anticoagulant containing buffer solutions the overall leucocyte concentration decreases and the yield is reduced.

Please keep in mind that added Carrier RNA will falsify the real genomic DNA content in photometric measurements.

Yield and quality of viral nucleic acids

The amount of purified nucleic acids in the **InviMag® Universal Kit/ KF96** procedure depends on the sample type, virus titer, sample source, transport, storage and age.

Yield and quality of the isolated viral nucleic acids is suitable for any pathogen detection system. The tests should be performed accordingly to manufacturers' specifications.

Different amplification systems vary in efficiency depending on the total amount of nucleic acid present in the reaction. Eluates derived by this kit will contain Carrier-RNA, which will greatly exceed the amount of the isolated NA.

Yields of viral nucleic acids isolated from biological samples are usually low concentrated and therefore almost impossible to determine photometrically.*

* Keep in mind that the Carrier-RNA (5 µg per 200 µl sample) will account for most of the present RNA.

The kit is suitable for downstream analysis with NAT techniques, for examples qPCR, RT-qPCR, LAMP, LCR. Diagnostic assays should be performed according to the manufacturer's instructions.

Quantitative RT-PCR is recommended for determination of viral RNA yield.

**) In Gel Electrophoresis and in Capillary Electrophoresis, RNA extracted with the provided kit looks like degraded cause the kit contains Carrier RNA, this is poly A RNA in fragments of 100 up to 1000 bases. The kit is not dedicated for applications using this kind of analysis.*

Protocol validation

The provided extraction protocols were intensively tested on a KingFisher Flex 96 instrument with the provided reagents and consumables. Typical results for the extraction of bacterial DNA, genomic DNA derived from blood, viral DNA and RNA are shown below. Actual results can vary, depending upon sample age, quality, type, and the species used.

Samples

For testing the isolation efficiency of bacterial DNA, frozen bacterial pellets from the gram-positive bacterium *Bacillus subtilis* were used in dilutions from 1 x 10^9 to 1 x 10^4. The bacteria were grown in an over-night culture and the derived cell pellets from 1 ml of this culture were stored at -20°C until further use. In all experiments a fresh pellet was used from the -20°C stock. The detection was done by an in-house *Bacillus subtilis* real-time PCR based detection assay performed on a Step One Plus Cycler (Applied Biosystems). For the extraction process 200 µl of the corresponding dilution was used, respectively.

For testing of genomic DNA derived from whole blood, 200 µl deeply frozen mammalian blood was used. The blood samples were either stabilized with EDTA or citrate.

a) Genomic DNA (human whole *blood*)

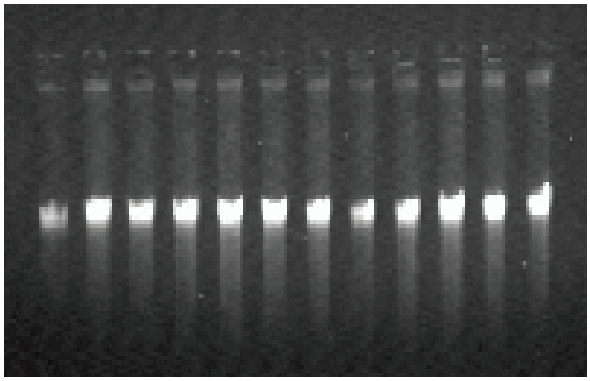


Fig. 4: Shown is a representative gel picture of genomic DNA (10 µl eluate / lane) derived from human whole blood separated on a 0.8% agarose gel. The staining was performed with ethidium bromide.

The average purity (OD260/OD320), calculated by photometric measurement, is usually in the range of 1.8-2.1. An average yield of about 3-4 µg DNA is derived from 200 µl human whole blood.

b) Bacterial Detection (Bacillus subtilis)

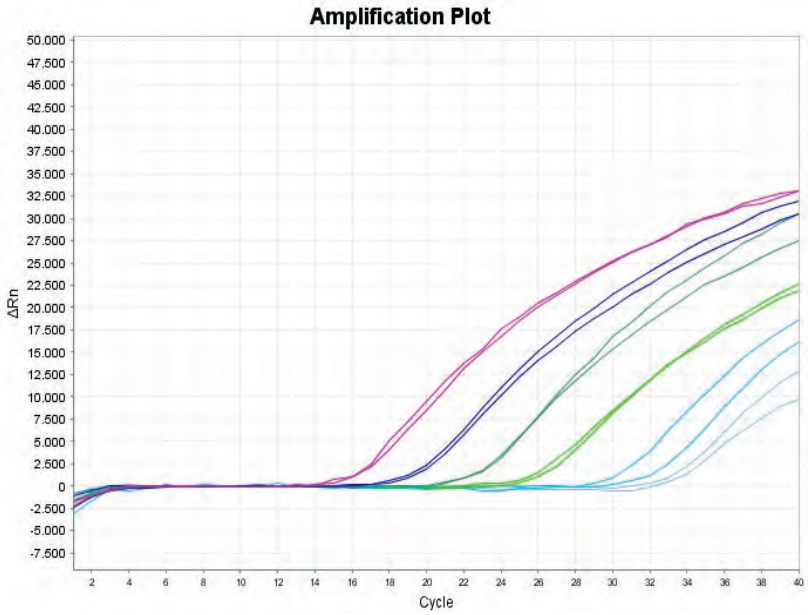


Fig. 1: Real-time PCR results from a representative RT-PCR run with *Bacillus subtilis* samples performed in dilutions (10^9 purple, 10^8 blue, 10^7 dark green, 10^6 light green, 10^5 cyan, 10^4 grey).

Sample	CT	CT SD
B.s. 10e9	16,545	0,199
B.s. 10e9	16,826	0,199
B.s. 10e8	19,740	0,163
B.s. 10e8	19,971	0,163
B.s. 10e7	23,201	0,112
B.s. 10e7	23,043	0,112
B.s. 10e6	26,334	0,348
B.s. 10e6	26,826	0,348
B.s. 10e5	32,639	1,295
B.s. 10e5	30,808	1,29
B.s. 10e4	34,363	0,349
B.s. 10e4	33,870	0,349

Tab. 1: The table shows the average Ct values and the corresponding standard deviation of *Bacillus subtilis* spiked samples derived by real-time PCR.

For testing of viral DNA and RNA, 200 µl plasma was spiked with 2 µl hCMV or 2 µl Influenza stock solution, respectively. The detection was done by an in-house real-time PCR for hCMV and Influenza, respectively. All real-time PCR's were performed on a Corbett Rotor-Gene 3000 or Step One Plus Cycler (Applied Biosystems).

c) RNA Virus Detection (*Influenza*)

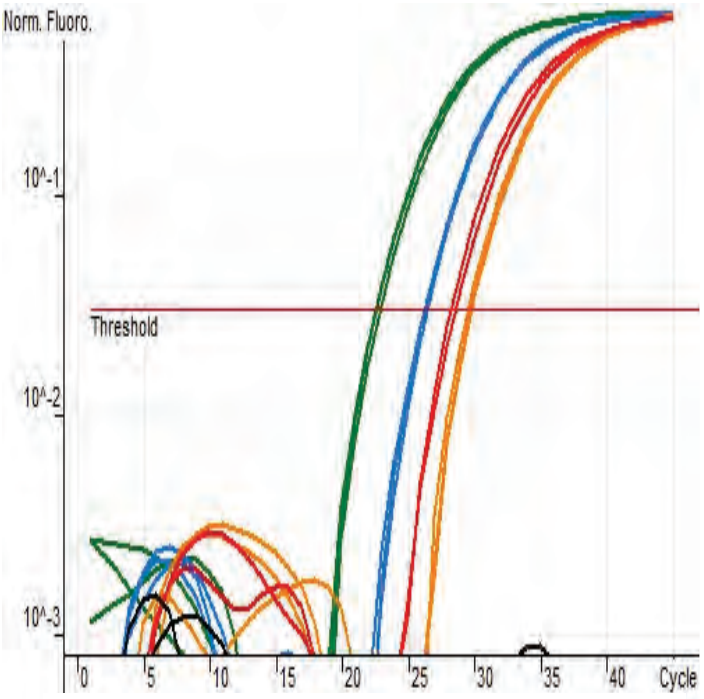


Fig. 2: Real-time PCR results from a representative RT-PCR run with *Influenza* spiked samples performed in dilutions (undiluted (green), 10^{-1} (blue), 10^{-2} (orange), PTC (red), NTC (black).

No.	Color	Name	Type	Ct
1		Influenza, undiluted	Sample	22,62
2		Influenza, undiluted	Sample	22,67
3		Influenza, undiluted	Sample	22,96
4		Influenza, 10^{-1}	Sample	26,30
5		Influenza, 10^{-1}	Sample	26,33
6		Influenza, 10^{-1}	Sample	26,53
7		Influenza 10^{-2}	Sample	29,90
8		Influenza 10^{-2}	Sample	29,80
9		Influenza 10^{-2}	Sample	29,61
10		PTC	Positive Control	28,65
11		PTC	Positive Control	28,32
12		NTC	Negative Control	
13		NTC	Negative Control	

Tab. 2: The table shows the estimated Ct values of *Influenza* spiked samples performed in dilutions and derived by real-time PCR.

d) DNA Virus Detection (*hCMV*)

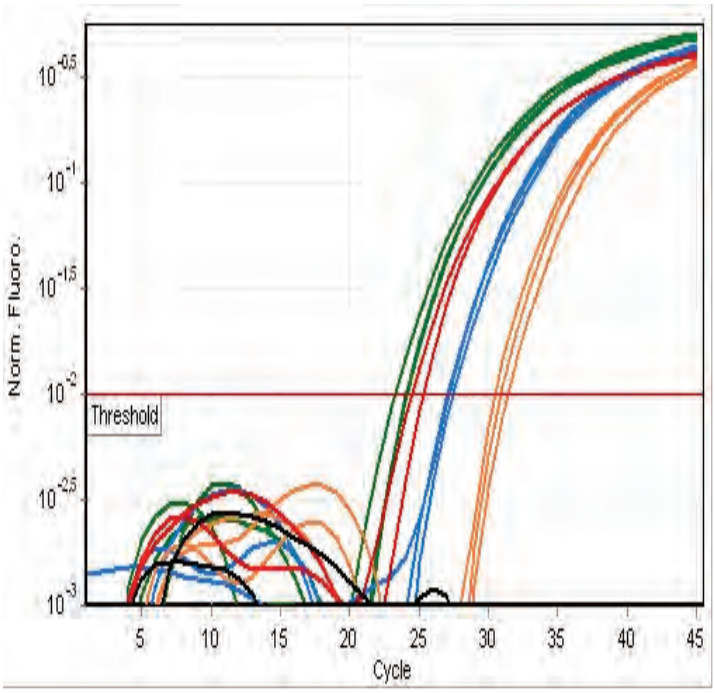


Fig. 3: Real-time PCR results from a representative RT-PCR run with *hCMV* spiked samples performed in dilutions (undiluted (green), 10^{-1} (blue), 10^{-2} (orange), PTC (red), NTC (black).

No.	Color	Name	Type	Ct
1		hCMV, undiluted	Sample	23,28
2		hCMV, undiluted	Sample	24,11
3		hCMV, undiluted	Sample	24,03
4		hCMV, 10^{-1}	Sample	27,21
5		hCMV, 10^{-1}	Sample	27,07
6		hCMV, 10^{-1}	Sample	27,47
7		hCMV, 10^{-2}	Sample	30,88
8		hCMV, 10^{-2}	Sample	30,46
9		hCMV, 10^{-2}	Sample	31,39
10		PTC	Positive Control	25,28
11		PTC	Positive Control	24,46
12		NTC	Negative Control	
13		NTC	Negative Control	

Tab. 3: The table shows the estimated Ct values of *hCMV* spiked samples performed in dilutions and derived by real-time PCR.

Important notes

Immediately upon receipt of the product, inspect the product and its components as well as the package for any apparent damages, correct quantities and quality. If there are any unconformities immediately notify STRATEC Molecular in writing with immediate effect upon inspection thereof. If buffer bottles are damaged, contact the STRATEC Molecular Technical Services or your local distributor. In case of liquid spillage, refer to “Safety Information” (see page 7). Do not use damaged kit components, because their use may lead to poor kit performance.

- Always change pipet tips between liquid transfers. We recommend the use of aerosol-barrier pipet tips to avoid cross-contaminations.
- All centrifugation steps are carried out at room temperature.
- When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles.
- Discard contaminated gloves immediately.
- Do not combine components of different kits unless the lot numbers are identical.
- Avoid microbial contamination of the kit reagents.
- We recommend working under laminar air-flow until the samples are lysed to minimize the risk of infections from potentially infectious material.

This kit should only be used by trained personnel.

Preparing reagents and buffers

Prior to each isolation
Before starting a run, bring all reagents to room temperature. Where necessary, gently mix and re-dissolve any precipitates by warming to 30°C until dissolved. Swirl gently to avoid foaming.

Proteinase K
Add described amount of ddH_2O (see table below) to needed tube or bottle of **Proteinase K**, mix thoroughly and store not needed **Proteinase K** at –20 °C. Dividing the **Proteinase K** into aliquots to avoid repeated freezing and thawing is recommended.

Lysozyme
Lysozyme should be prepared directly before starting the isolation. Solve the lyophilized Lysozyme within the bottle of Lysozyme Buffer. Mix thoroughly until all of the Lysozyme is solved. Store not directly used Lysozyme solution in aliquots at –20°C and avoid repeated freezing and thawing.

Wash Buffer I and Wash Buffer II
Before use add the described volume of 96-100% ethanol to the bottle with Wash Buffer I and II as described below. After adding the ethanol mix shortly and keep the bottles always firmly closed!

Wash Buffer HLT
Before use add the described volume Isopropanol to the bottle with Wash Buffer HLT as described below. After adding mix shortly and keep the bottles always firmly closed!

1 x 96 extractions:
Add 60 ml of abs. 99.7% Isopropanol to the bottle **Wash Buffer HLT** and mix thoroughly Add 90 ml of 96-100% ethanol to the bottle **Wash Buffer M** and mix thoroughly. Add 105 ml of 96-100% ethanol to the bottle **Wash Buffer II** and mix thoroughly. Add the provided amount of **Lysozyme** to the bottle with the **Lysozyme Buffer** and mix it thoroughly. Resuspend each tube **Carrier-RNA** in 1.2 ml RNAse free water. Mix thoroughly until completely dissolving.Resuspend each tube **Proteinase K** in 1.1 ml RNAse free water, mix thoroughly until completely dissolving and store at -20°C! Add 30 ml 99.7% Isopropanol (molecular biologic grade) to the empty bottle labeled with “Binding Solution”

5 x 96 extractions:
Add 240 ml of abs. 99.7% Isopropanol to the bottle **Wash Buffer HLT** and mix thoroughly Add 450 ml of 96-100% ethanol to the bottle **Wash Buffer M** and mix thoroughly. Add 420 ml of 96-100% ethanol to the bottle **Wash Buffer II** and mix thoroughly. Add the provided amount of **Lysozyme** to the bottle with the **Lysozyme Buffer** and mix it thoroughly. Resuspend the **Carrier-RNA** in 15 ml RNAse free water. Mix thoroughly until completely dissolving. Resuspend the **Proteinase K** in 10.5 ml RNAse free water, mix thoroughly until completely dissolving and store at -20°C! Add 120 ml 99.7% **Isopropanol** (molecular biologic grade) to the empty bottle labeled with “Binding Solution”

Reagents and equipment to be supplied by user

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDS) on our webpage **www.stratec.com**.

- o Microcentrifuge ≥ 9.300 x *g* (≥ 10.000 rpm), optional*
- o Ethanol (96 - 100%)
- o 1.5 ml reaction tubes, optional
- o Measuring cylinder (250 ml)
- o Disposable gloves
- o Pipet with tips (we highly recommend to use filter tips only)
- o 15 or 50 ml reaction tubes, optional
- o Isopropanol*

*)The **InviMag® Universal Kit/ KF96** is validated with 2-Propanol; Rotipuran >99.7%, p.a., ACS, ISO (Order no. 6752) from **Carl Roth.**

Possible suppliers for Isopropanol:

Carl Roth
2-Propanol
Rotipuran >99.7%, p.a., ACS, ISO
Order no. 6752

Applichem
2-Propanol für die Molekularbiologie
Order no. A3928

Sigma
2-Propanol
Order no. 59304-1L-F

Important indications

Preparing RNA: When preparing RNA, work quickly during the manual steps of the procedure. Special care should be taken to avoid contaminations of Elution Buffer M with DNases/ RNases.

Storing samples: Frozen serum or plasma samples should not be thawed more than once. Repeated freezing and thawing cycles may lead to denaturation and precipitation of proteins, resulting in reduced titers and therefore reduced yields.

Carrier RNA: Carrier RNA serves two purposes: It enhances the binding of nucleic acids to the beads, especially if there are only very few target molecules present in the sample. Furthermore, the addition of Carrier RNA reduces the chance of nucleic acid degradation in the rare event that RNase or DNase molecules are not denaturated completely by the Lysis Buffer.

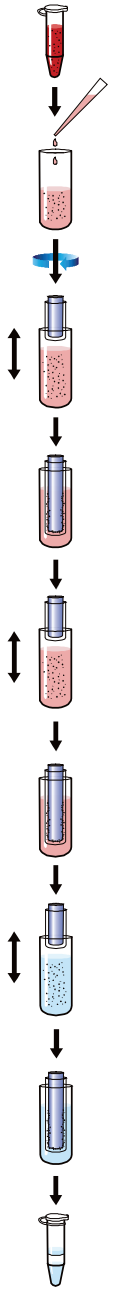
Internal Controls: The use of an internal control is recommended when using the **InviMag® Universal Kit/ KF96** in combination with diagnostic amplification systems. Internal controls should be added directly to the lysis mixture during the pause step where the Binding Solution and beads are added. Do not add any controls to the sample or to the lysis mixture until the lysis step is complete. In rare cases the controls may be degraded by DNases/RNases present in the sample. Alternatively, internal controls can be prepared directly in the Carrier RNA tube. In that case, the amount of added water to the Carrier RNA has to be reduced. Do not exceed the final volume of 1.2 ml / Carrier RNA tube.

Scheme of the InviMag® Universal Kit/ KF96

Please read protocols prior the start of the preparation carefully.

Transfer 200 µl **Lysis Buffer HLT** and 200 µl **sample** into a cavity of a 2 ml Deep Well Plate (refers as "Lysis Plate"). Add 20 µl Proteinase K and 20 µl Carrier RNA (optional for genomic DNA). For a bacterial DNA preparation 20 µl of Lysozyme should be added in the first step. Continue with the respective lysis protocol
Prefill all plates with required buffers and appropriate volumes.

Tip Plate:	Insert the KF96 Tip Comb for DW magnets on a Tip Plate*
Lysis Plate:	See "lysis procedures" (page 17) for respective protocol
Washing Plate_1:	Add 900 µl **Wash Buffer HLT** to a 2.0 ml Deep Well Plate
Washing Plate_2:	Add 900 µl **Wash Buffer M** to a 2.0 ml Deep Well Plate
Washing Plate_3:	Add 1000 µl **Wash Buffer II** to a 2.0 ml Deep Well Plate
Elution Plate:	Add 100 µl **Elution Buffer M** to the Elution Plate (same size as Tip Plate)



Please read the protocols carefully prior to the start of the preparation procedure!

The following steps are performed on the KingFisher instrument:

Lysis of the sample

After lysis, during the pause step, please add **230 µl Binding Solution** and **20 µl SNAP Solution**

Important: *If an internal extraction control should be used, please add it to the reaction mixture at this step.*

Nucleic acids bind to magnetic particles

Washing of the particle fixed nucleic acids

Magnetic separation

Elution of nucleic acids

Magnetic Separation

Pure nucleic acids

* Elution Plates and Tip Plates are identically. Use one provided Elution Plate as a Tip Plate.

Lysis procedures

For easier handling we recommend to prepare a master mix consisting of Lysis Buffer HLT, Proteinase K and if required Carrier RNA. When preparing the **Master Mix** it is recommended to use a volume of 5 % greater than that required.

Attention: *Please be aware, that you have to prepare the Master Mix shortly before carrying out the purifications adapted to the number of samples that will be processed.*
Longer incubation will decrease the activity of the Proteinase K

Preparation of a Master mix

Number of samples	Amount of **Lysis Buffer HLT**	Amount of **Carrier RNA**	Amount of **Proteinase K**
	200 µl / sample	20 µl / samples	20 µl / samples
8	1.7 ml	170 µl	170 µl
16	3.4 ml	340 µl	340 µl
24	5.0 ml	500 µl	500 µl
32	6.7 ml	670 µl	670 µl
40	8.4 ml	840 µl	840 µl
48	10.0 ml	1000 µl	1000 µl
56	11.8 ml	1180 µl	1180 µl
64	13.4 ml	1340 µl	1340 µl
72	15.1 ml	1510 µl	1510 µl
80	16.8 ml	1680 µl	1680 µl
88	18.5 ml	1850 µl	1850 µl
96	20.2 ml	2020 µl	2020 µl

Protocol 1: Simultaneous isolation of total nucleic acids from cell- free body fluids or of DNA from blood (genomic DNA)

Please read the protocols carefully prior to the start of the preparation procedure!

Important Note: *The protocol is optimized for a sample volume of 200 µl. For smaller samples volumes than 200 µl please fill up to a total volume of 200 µl with ddH_2O or PBS.*

Transfer 200 µl sample into a cavity of a 2 ml Deep Well Plate (refers as “Lysis Plate”).

A) Add 200 µl Lysis Buffer HLT, 20 µl Proteinase K and 20 µl Carrier RNA to each sample. If genomic DNA shall be prepared, the addition of Carrier RNA is optional.

or

B) Add 240 µl Master Mix (as described at point Lysis Procedure, page 17) to each sample. If genomic DNA shall be prepared, the addition of Carrier RNA to the Master Mix is optional.

Prefill the remaining plates with required buffers and appropriate volumes and continue with the loading of the system (see “Starting a Run”, page 23).

Protocol 2: Simultaneous isolation of nucleic acids (DNA/RNA) from swab material

Please read the protocols carefully prior to the start of the preparation procedure!

2.1 Use of swabs

A) Add 200 µl Lysis Buffer HLT, 20 µl Proteinase K and 20 µl Carrier RNA into each cavity of a 2 ml Deep Well Plate (refers as “Lysis Plate”). If genomic DNA shall be prepared, the addition of Carrier RNA is optional. For the preparation of bacterial DNA, we recommend to use 20 µl Lysozyme for an improved lysis. Add the Lysozyme directly to the Lysis Plate before adding samples or other reagents.

or

B) Add 240 µl Master Mix (as described at point Lysis Procedure, page 17) to into each cavity of a 2 ml Deep Well Plate (refers as “Lysis Plate”).

If genomic DNA shall be prepared, the addition of Carrier RNA to the Master Mix is optional.

Insert the swab into the cavity of the Lysis Plate. Incubate for 5 - 10 min at RT and stir occasionally. After incubation, remove the swab and squeeze it out inside the cavity to remove residual liquid and then discard the swab.

Prefill all remaining plates with the required buffers and appropriate volumes and continue with the loading of the system (see “Starting a Run”, page 23).

2.2. Usage of rinsed liquid from swab

a) the sample <u>will also be used</u> for cultivation

Cut off the relevant part of the swab and transfer it into a RNAse/DNAse-free 2 ml tube. Add 300 µl physiological saline solutions to the swab and vortex intensely for 2 - 3 min and incubate for 10 min at RT. Use an aliquot for cultivation.

For the preparation of bacterial DNA, we recommend to use 20 µl Lysozyme for an improved lysis. Add the 20 µl Lysozyme directly to the Lysis Plate before adding samples or other reagents or Master Mix.

Transfer than 200 µl of the rinse liquid into a cavity of a 2 ml Deep Well Plate (refers as “Lysis Plate”)

A) Add 200 µl Lysis Buffer HLT, 20 µl Proteinase K and 20 µl Carrier RNA to each sample. If genomic DNA shall be prepared, the addition of Carrier RNA is optional.

or

B) Add 240 µl Master Mix (as described at point Lysis Procedure, page 17) to each sample. If genomic DNA shall be prepared, the addition of Carrier RNA to the Master Mix is optional.

For bacterial DNA: don’t forget to add the Lysozyme at first in the plate.

Prefill all remaining plates with the required buffers and appropriate volumes and continue with the loading of the system (see “Starting a Run”, page 23).

b) the sample <u>will not be used</u> for cultivation

Cut off the relevant part of the swab and transfer this part into a RNase- and DNAse-free 2 ml tube. Add 300 µl RNase-free water to the swab and vortex intensely for 3 min. Optional, incubate for 3 min at 95°C.

For the preparation of bacterial DNA, we recommend to use 20 µl Lysozyme for an improved lysis. Add the Lysozyme directly to the Lysis Plate before adding samples or other reagents.

Transfer than 200 µl of the rinsed liquid into a cavity of a 2 ml Deep Well Plate (refers as “Lysis Plate”)

A) Add 200 µl Lysis Buffer HLT, 20 µl Proteinase K and 20 µl Carrier RNA to each sample. If genomic DNA shall be prepared, the addition of Carrier RNA is optional.

Or

B) Add 240 µl Master Mix (as described at point Lysis Procedure, page 17) to each sample. If genomic DNA shall be prepared, the addition of Carrier RNA to the Master Mix is optional.

For bacterial DNA: don't forget to add the Lysozyme at first in the plate (see above).

Prefill all remaining plates with the required buffers and appropriate volumes and continue with the loading of the system (see "Starting a Run", page 23).

Protocol 3: Simultaneous isolation of nucleic acids (DNA and RNA) from tissue biopsies

Please read the protocols carefully prior to the start of the preparation procedure!

For the preparation of bacterial DNA, we recommend to use 20 µl Lysozyme for an improved lysis. Add the Lysozyme directly to the Lysis Plate before adding samples or other reagents

Transfer 1 - 10 mg from the tissue biopsy sample into a cavity of a 2 ml Deep Well Plate (refers as "Lysis Plate") and add 200 µl distilled water, 200 µl Lysis Buffer HLT, 20 µl Carrier RNA and 20 µl Proteinase K to each sample or add 240 µl Master Mix (as described at point Lysis Procedure, page 16.) to each sample. If genomic DNA shall be prepared, the addition of Carrier RNA to the Master Mix or to the sample is optional.

Prefill the remaining plates with required buffers and appropriate volumes and continue with the loading of the system (see "Starting a Run", page 23).

Protocol 4: Isolation of DNA from bacteria pellets (up to 1x 10^9 bacterial cells) or from non-viscous tracheal secrete of BAL

Please read the protocols carefully prior to the start of the preparation procedure!

For sample preparation use an aliquot from the bacterial culture and centrifuge the sample at 9.300 x *g* (10.000 rpm) for 3 min. Discard the supernatant without disturbing the bacterial pellet.

For an improved lysis add 20 µl Lysozyme directly to the Lysis Plate before adding samples or other reagents.

Resuspend the bacterial pellet in 200 µl distilled water or PBS and transfer the sample into a cavity of a 2 ml Deep Well Plate (refers as "Lysis Plate").

A) Add 200 µl Lysis Buffer HLT, 20 µl Carrier RNA and 20 µl Proteinase to each cavity

   or

B) Add 240 µl Master Mix (as described at point Lysis Procedure, page 17) to each sample.

Don't forget to add the Lysozyme at first in the plate (see above).

Prefill the remaining plates with required buffers and appropriate volumes and continue with the loading of the system (see "Starting a Run", page 23).

Protocol 5: Simultaneous isolation of total nucleic acids from sputum

Please read the protocols carefully prior to the start of the preparation procedure!

Transfer 150 µl from the sputum sample into a RNase/DNAse-free tube and add 150 µl NAC Buffer (order number: 1033221100) or saturated acetylcysteine (ACC) solution to the sample (ratio sample to buffer must be 1:1).

Incubate the mixture for 10 min at 95°C to reduce the viscosity.

For viral NA preparation, the temperature is not disturbing the next steps.

Prepare the 2 ml Deep Well Plate (refers as “Lysis Plate”) with Lysozyme for the preparation of bacterial DNA. During this time the temperature of the sample will decrease and prevent an inactivation of Lysozyme. Use 20 µl Lysozyme for an improved lysis. Add the 20 µl Lysozyme directly to the cavities of the Lysis Plate before adding samples or other reagents.

Transfer 200 µl from the sample mixture into the cavity of a 2 ml Deep Well Plate (refers as “Lysis Plate”).

A) Add 200 µl Lysis Buffer HLT, 20 µl Carrier RNA and 20 µl Proteinase K. If only genomic DNA is processed the addition of Carrier RNA is optional.

or

B) Add 240 µl Master Mix (as described at point Lysis Procedure, page 17) to each sample.

Prefill the remaining plates with required buffers and appropriate volumes and continue with the loading of the system (see “Starting a Run”, page 23).

Protocol 6: Simultaneous isolation of viral nucleic acids (DNA and RNA) from slimy tracheal secretes or BAL

Please read the protocols carefully prior to the start of the preparation procedure!

Non viscous samples:

Transfer 1 ml of tracheal secret or BAL into a RNase/DNAse-free tube and centrifuge at 9.300 x *g* (10.000 rpm) for 3 min. Discard the supernatant without disturbing the bacterial pellet: Resuspend the bacterial pellet in 200 µl distilled water or PBS and transfer the sample into a cavity of a 2 ml Deep Well Plate (refers as “Lysis Plate”).

A) Add 200 µl Lysis Buffer HLT, 20 µl Carrier RNA and 20 µl Proteinase K. For the preparation of bacterial DNA, we recommend to use 20 µl Lysozyme for an improved lysis. Add the 20 µl Lysozyme each cavity directly into the Lysis Plate before adding samples or other reagents.

or

B) Add 240 µl Master Mix (as described at point Lysis Procedure, page 16.) to each sample. For the preparation of bacterial DNA, we recommend to use Lysozyme for an improved lysis. Use the same volumes as Proteinase K shown in the table of Lysis procedures (page 17) for preparation of the Master Mix.

Prefill the remaining plates with required buffers and appropriate volumes and continue with the loading of the system (see “Starting a Run”, page 23).

Viscous sample

Transfer 1 ml of tracheal secrete or BAL into a RNase- and DNAse-free tube and add 1 ml NAC Buffer (order number: 1033221100) or saturated acetylcysteine (ACC) solution to the sample (ratio sample to buffer must be 1:1)

Incubate the mixture for 10 min at 95°C to reduce the viscosity and centrifuge at 9.300 x *g* (10.000 rpm) for 3 min. Discard the supernatant without disturbing the bacterial pellet directly.

Resuspend the bacterial pellet in 200 µl distilled water or PBS.

Prepare the 2 ml Deep Well Plate (refers as “Lysis Plate”) with Lysozyme for the preparation of bacterial DNA. Use 20 µl Lysozyme for an improved lysis. Add the 20 µl Lysozyme directly to the cavities of the Lysis Plate before adding samples or other reagents.

Transfer the 200 µl sample now into a cavity of a 2 ml Deep Well Plate (refers as “Lysis Plate”).

A) Add 200 µl Lysis Buffer HLT, 20 µl Carrier RNA and 20 µl Proteinase K

or

B) Add 240 µl Master Mix (as described at point Lysis Procedure, page 17) to each sample.

It is also possible to add the Lysozyme to the Master Mix. Use the same volumes as Proteinase K shown in the table of Lysis procedures (page 17) for preparation of the Master Mix.

Prefill the remaining plates with required buffers and appropriate volumes and continue with the loading of the system (see “Starting a Run”, page 23).

Protocol 7: Simultaneous isolation of viral nucleic acids from stool samples

Please read the protocols carefully prior to the start of the preparation procedure!

Transfer 100 µl stool sample into a 2 ml tube and dilute the sample 1:10 with RNase-free water. Vortex the sample for 30 s followed by a 1 min centrifugation step at 12.000 x *g* (13.000 rpm).

Transfer 200 µl virus containing supernatant into the cavity of a 2 ml Deep Well Plate (refers as “Lysis Plate”).

A) Add 200 µl Lysis Buffer HLT, 20 µl Carrier RNA and 20 µl Proteinase K.

or

B) Add 240 µl Master Mix (as described at point Lysis Procedure, page 17) to each sample.

Prefill the remaining plates with required buffers and appropriate volumes and continue with the loading of the system (see “Starting a Run”, page 23).

Protocol 8: Isolation of bacterial DNA from stool samples

Please read the protocols carefully prior to the start of the preparation procedure!

Transfer 100 µl stool sample into a 2 ml tube and add 300 µl RNase-free water. Vortex the sample for 30 s followed by a 30 s centrifugation step at 3.000 rpm. (1.000 x *g*)

Prepare the 2 ml Deep Well Plate (refers as “Lysis Plate”) with Lysozyme for the preparation of bacterial DNA. Use 20 µl Lysozyme for an improved lysis. Add the 20 µl Lysozyme directly to the cavities of the Lysis Plate before adding samples or other reagents.

Transfer 200 µl of the bacteria containing supernatant into the cavity of a 2 ml Deep Well Plate (refers as “Lysis Plate”).

A) Add 200 µl Lysis Buffer HLT, 20 µl Carrier RNA and 20 µl Proteinase K.

or

B) Add 240 µl Master Mix (as described at point Lysis Procedure, page 17) to each sample.

It is also possible to add the Lysozyme to the Master Mix. Use the same volumes as Proteinase K shown in the table of Lysis procedures (page 17) for preparation of the Master Mix.

Prefill the remaining plates with required buffers and appropriate volumes and continue with the loading of the system (see “Starting a Run”, page 23).

Starting a Run

I. Preliminary Steps to process the sample onto the KingFisher System

Important: *For working with the KF96 / KFflex96 instrument, please carefully read the manufacturer´s manual before using the system for the first time!*

1. Switch on the KF96 / KFflex96 instrument

Tip Plate: Place the KF96 Tip Comb for DW magnets on an Elution Plate (Tip Plate).

Note*: Use one provided Elution Plate as Tip Plate. These are identical.*

2. Prefill the all Deep Well Plates with the required buffers and appropriate volumes

Important: *Mix the bottle with the **SNAP Solution** by vigorously vortexing before usage!*

Lysis Plate: see the corresponding isolation protocol (chapter “Lysis procedures”, page 17)

Washing plate_1: Add 900 µl **Wash Buffer HLT** into the cavities of a Deep Well Plate

Washing plate_2: Add 900 µl **Wash Buffer M** into the cavities of a Deep Well Plate

Washing plate_3: Add 1000 µl **Wash Buffer II** into the cavities of a Deep Well Plate

Elution Plate: Add 100 µl **Elution Buffer M** into the cavities of a Elution Plate

3. Choose the assay file”**InviMag_Universal_KF96”** or **“InviMag_Universal_KFflex96”** on the display of the instrument – depends on the used instrument - and press the “START” button.
4. Insert the prefilled plates onto the right position of the KingFisher surface by following the specification shown on the display. Confirm every step by pressing the “START” button.
5. If all prefilled plates are loaded into the system press the “START” button again to start the assay file.
6. After the lysis steps, a pause will occur and 230 µl **Binding Solution** and 20 µl **SNAP Solution** have to be added to each used cavity of the Lysis Pate. If extraction controls should be used please add them at this step too. Alternatively, the internal control can be added to the Carrier-RNA tube (see page 15).
7. Reinsert the plate into the instrument (watch out for correct plate orientation) and continue the run by pressing the “START” button. The instrument will now finish the purification process without any further user interaction.

II. The following steps run automatically on the KingFisher System

Specific sample preparation

1. **Lysis**
   Lysis is performed at elevated temperature for 15 min. After lysis, the instrument will be paused and the user has to add **230 µl Binding Solution** and **20 µl SNAP Solution** (magnetic beads). Internal extraction controls should be added during this pause step too.

2. **Binding of the nucleic acids**
   Binding step for 5 min. SNAP separation. Transportation of the SNAP bound nucleic acids into Washing Plate 1.

3. **First Washing Step**
   Sample washing for 5 min. SNAP separation. Transportation of the SNAP bound nucleic acids into the Washing Plate 2.

4. **Second Washing Step**
   Sample washing for 4 min. SNAP separation. Transportation of the SNAP bound nucleic acids into the Washing Plate 3.

5. **Third Washing and Drying Step**
   Sample washing for 3 min. SNAP separation. Air drying of the SNAP bound nucleic acids outside the plate for 5 min. Transportation of the SNAP into the Elution Plate.

6. **Elution of the nucleic acids**
   Incubation of the SNAP bound nucleic acids into the Elution Plate for 10 min by mixing at elevated temperature. SNAP separation. The SNAPs without the bound nucleic acids are afterwards automatically discarded into the wells of Washing Plate_3 (disposal).

Important Notes: *After finishing the extraction protocol, the Elution Plate contains the extracted nucleic acids. Store the nucleic acids at adequate conditions. We recommend transferring the extracted nucleic acids into 1.5 ml Receiver Tubes (provided) and store them at -20°C or –80°C.*

If the extracted nucleic acids contain carry-over of magnetic particles, transfer them into a 1.5 ml reaction tube and centrifuge at max. speed for 1 min. Then transfer the nucleic acids containing supernatant into the provided Receiver Tubes.

The eluted nucleic acids are ready-to-use in different downstream applications.

For self-programming of the KF96 and KFflex96 instrument

Reagent info

Tip Plate		KingFisher 96 KF plate	
Name	Well volume [µl]	Total reagent volume [µl]	Type
-	-	-	-

Lysis Plate		Microtiter DW 96 plate	
Name	Well volume [µl]	Total reagent volume [µl]	Type
Sample	200	-	Sample
Lysis Buffer HLT	200	-	Reagent
Proteinase K	20	-	Reagent
Carrier-RNA	20	-	Reagent
Lysozyme (if required)	20	-	Reagent

Wash Plate 1		Microtiter DW 96 plate	
Name	Well volume [µl]	Total reagent volume [µl]	Type
Wash Buffer HLT	900	-	Reagent

Wash Plate 2		Microtiter DW 96 plate	
Name	Well volume [µl]	Total reagent volume [µl]	Type
Wash Buffer M	900	-	Reagent

Wash Plate 3		Microtiter DW 96 plate	
Name	Well volume [µl]	Total reagent volume [µl]	Type
Wash Buffer II	1000	-	Reagent

Elution Plate		KingFisher 96 KF plate	
Name	Well volume [µl]	Total reagent volume [µl]	Type
Elution Buffer M	100	-	Reagent

Dispensed reagents

Lysis Plate		Microtiter DW 96 plate	
Name	Step	Well volume [µl]	Total reagent volume [µl]
Isopropanol	Adjust Binding Condition	230	-
SNAP Solution	Adjust Binding Condition	20	-

Steps data

	Tip1	96 DW tip comb	
	Pick-Up	Tip Plate	
	Lysis Step	Lysis Plate	
	Beginning of step	Precollect	No
		Release beads	Yes
	Mixing / heating:	Mixing time, speed	00:15:00, Medium
		Heating temperature [°C]	75
		Preheat	Yes
	End of step	Postmix	No
		Collect beads	No
	Adjust Binding Condition	Lysis Plate	
		Message	Add Isopropanol + SNAPs
		Dispensing volume [µl]	250
	Reagent(s)	Name	Isopropanol
		Volume [µl]	230
		Name	SNAP Solution
		Volume [µl]	20
	Binding Step	Lysis Plate	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Fast
	Mixing / heating:	Mixing time, speed	00:05:00, Medium
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	4
		Collect time [s]	5
	Washing Step 1	Wash Plate 1	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Fast
	Mixing / heating:	Mixing time, speed	00:05:00, Fast
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	4
		Collect time [s]	5
	Washing Step 2	Wash Plate 2	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Fast
	Mixing / heating:	Mixing time, speed	00:04:00, Fast
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	4
		Collect time [s]	5

	Washing Step 3	Wash Plate 3	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Fast
	Mixing / heating:	Mixing time, speed	00:03:00, Fast
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	4
		Collect time [s]	5
	Drying Step	Wash Plate 3	
		Dry time	00:05:00
		Tip position	Outside well / tube
	Elution Step	Elution Plate	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Medium
	Mixing / heating:	Mixing time, speed	00:10:00, Medium
		Heating temperature [°C]	65
		Preheat	Yes
	End of step	Postmix	No
		Collect count	4
		Collect time [s]	10
	Bead Removal Step	Wash Plate 3	
		Release time, speed	00:00:30, Fast
	Leave	Tip Plate	

Troubleshooting

Problem	Probable cause	Comments and suggestions
Low amount of extracted DNA	Insufficient lysis	Increase lysis time, but prevent too long lysis time because this also decreases the yield Reduce amount of starting material
	Incomplete elution	Increase the volume of **Elution Buffer M.** ensure that the **Elution Buffer M** is transferred into the right position; change the modified volume in the provided assay file too
	Low amount of SNAP Solution	Mix **SNAP Solution** vigorously before use
Low concentration of extracted DNA	Too much Elution Buffer	Elute the DNA with in a lower volume of **Elution Buffer R**. Change the modified volume in the run file too.
	Incorrect storage of starting material	Ensure that the storage of starting material was correct. Avoid repeated thawing and freezing cycles of the sample material
	Incorrect Wash Buffers	Ensure, that the correct amount of ethanol/isopropanol is added to the **Wash Buffers** and stored correctly
Degraded DNA	Incorrect storage of starting material	Ensure that the storage of starting material was correct
	Old material	Ensure that the starting material is stored at appropriate conditions (–20°C/-80°C) avoid multiple thawing and freezing cycles of the material
DNA does not perform well in downstream-applications (e.g. real-time PCR or PCR)	No PCR result for genomic DNA	Due to the very gentle isolation procedure it may happen that isolated genomic DNA forms a ball. To overcome this, the primary PCR denaturation step at 95°C should be prolonged to 5 min
	Ethanol carryover during elution	Increase drying time for removal of ethanol in the assay file
	Salt carry-over during elution	Check the **Wash Buffers** for salt precipitates. If there are any precipitates visible, solve them by carefully warming up to 30°C Ensure that the **Wash Buffers** are equilibrated at room temperature
Eluted DNA is brownish colored	Small part of the magnetic particles are left in the elution	Centrifuge at full speed for 1 min and transfer supernatant to a new tube

Problem	Probable cause	Comments and suggestions
Low amount of extracted RNA	Insufficient lysis	Increase lyses time, but prevent too long lyses time because this decrease the yield Reduce amount of starting material
	Incomplete elution	Use a higher volume of **Elution Buffer M**. Ensure you pipet the **Elution Buffer M** with the correct volume to the right position. Change the modified volume in the provided assay file too
	Low amount of SNAP Solution	Mix **SNAP Solution** thoroughly before pipetting to the Deep Well Plate
Low concentration of extracted RNA	Too much Elution Buffer R	Elute the RNA with lower volume of Elution Buffer R. Change the modified volume in the run file too
	Incorrect storage of starting material	Ensure that the storage of starting material was correctly avoid repeated thawing and freezing cycles of the sample material
	Incorrect Wash Buffers	Ensure that the correct amount of ethanol/ isopropanol is added to the **Wash Buffers**
Degraded RNA	Incorrect storage of starting material	ensure that the storage of starting material was correctly Avoid multiple thawing and freezing of the sample material
	Old material	Ensure that the starting material is fresh or stored at appropriate conditions (-20°C / -80 C) Avoid multiple thawing and freezing of the material
RNA does not perform well in downstream-applications (e.g. real-time RT-PCR or RT-PCR)	Ethanol carryover during elution	increase drying time for removing of ethanol in the assay file
	Salt carryover during elution	Check the **Wash Buffers** for salt precipitates. If there are any precipitates visible, solve them by carefully warming up to 30°C Ensure that the **Wash Buffers** are equilibrated at room temperature
Eluted RNA is brownish colored	Small parts of the magnetic particles are left in the elution	Centrifuge at full speed for 1 min and transfer supernatant into a new tube

Appendix

KingFisher BindIt Software 3.2 or higher versions

BindIt software 3.2 or higher versions were and may be used to create assay files for the KFmL, KF96/KFflex96 or KF-Duo instruments. The provided assay file(s) can either be transferred onto the corresponding workstation(s) or be started directly from within the BindIt software after assay import. Please keep in mind, that assay(s) run from within the BindIt software are not stored in the workstation memory.

Important: *Be advised that BindIt SW 3.2 or higher versions use a new unique file extension. Therefore, it is not possible to import assay files created with BindIt 3.2 or higher versions into older BindIt software versions! Please ask your local Thermo Scientific distributor for a software update.*

Note*:* *When creating assay files for usage with KingFisher instruments in combination with Microtiter Deep Well plates (e.g. Thermo Electron), it is essential to use the KingFisher software 3.2 or higher versions for assay development because this software version includes the correct adjustments for the microtiter plate. It is highly recommended to use Thermo Microtiter Deep Well plates with KF96 / KFflex96 / KF-Duo workstations to ensure the best purification result.*

Minimum system requirements for BindIt Software 3.2 or higher versions

PC requirements	
Supported operating systems	MS Windows XP Pro with SP3, Windows Vista SP2, Windows 7
Disk space	500 MB free disk space
Processor	Intel Pentium ≥ 1 GHz
Memory	1 GB RAM
Serial ports available	1 (for KFmL connection)
USB ports available	1 (for KF96 / KFflex96 / KFDuo connection)
Pointing device	Mouse or equivalent is required
CD-ROM drive	1
Monitor / color settings	XVGA monitor with at least 1024x768 resolution and at least a 16-bit color environment

If the actual Windows Service Packs are not installed on the corresponding lab computer, they can be downloaded from the Microsoft web pages: http://www.microsoft.com/

General notes on handling DNA

Nature of DNA
The length and delicate physical nature of DNA requires careful handling to avoid damage due to shearing and enzymatic degradation. Other conditions that affect the integrity and stability of DNA include acidic and alkaline environments, high temperature, and UV irradiation. Careful isolation and handling of high molecular weight DNA is necessary to ensure it will work well in various downstream applications. Damaged DNA could perform poorly in applications such as genomic Southern blotting, long-template PCR, and construction of cosmid libraries.

Handling fresh and stored material before the extraction of DNA
For the isolation of genomic DNA from cells or tissues, use either fresh samples or samples that have been quickly frozen in liquid nitrogen and stored at -80°C. This procedure minimizes degradation of crude DNA by limiting the activity of endogenous nucleases.

Storage of DNA
Store DNA at 2 - 8°C. Storage of genomic DNA at -20°C may cause shearing, particularly if the DNA is exposed to repeated freezing and thawing cycles.

Drying, dissolving and pipetting DNA
Avoid overdrying genomic DNA after ethanol precipitation. It is better to air-dry DNA than to use a vacuum, although vacuum drying can be used with caution.

To help dissolve the DNA, carefully invert the tubes several times after adding buffer and tap the tube gently on the side. Alternatively incubate the DNA in buffer overnight at 2 - 8°C. Minimize vortexing of genomic DNA because this can cause shearing.

Avoid vigorous pipetting. Pipetting genomic DNA through small tip openings can cause shearing or nicking. One way to decrease shearing of genomic DNA is to use special tips that have wide openings designed for pipetting genomic DNA. Regular pipette tips pose no problem for plasmid and other small DNA.

Quantification
Quantification of DNA and RNA from this assay must be done by means of qPCR or Reverse Transcriptase qPCR. All other methods will be disturbed by the included Carrier RNA as well as DNA or RNA which will be co-purified.

General notes on handling RNA

RNA is far less stable than DNA. It is very sensitive to degradation by endogenous RNAses in the biological material and exogenous RNAses which are permanently present everywhere in the lab. To achieve satisfactory qualitative and quantitative results in RNA preparations, contaminations with exogenous RNAses have to be reduced to a minimum. Avoid handling bacterial cultures, cell cultures, or other biological sources of RNases in the same lab where the RNA purification is carried out.

All glassware must be RNAse free. Therefore, the glassware should be cleaned with detergent, thoroughly rinsed and oven baked at 240°C for four or more hours before use. Autoclaving only will not completely inactivate many RNAses. Oven baking will both inactivate RNAses and ensure that no other nucleic acids (such as plasmid DNA) are present on the surface of the glassware. You can also clean glassware with 0.1% DEPC (diethyl pyrocarbonate). The glassware must react 12 hours at 37°C and should then be autoclaved or heated to 100°C for 15 min to inactivate residual DEPC.

- **Electrophoresis tanks** should be cleaned with detergent solution (e.g. 0.5% SDS), thoroughly rinsed with RNase free water, rinsed with ethanol and allowed to air-dry.
- **Non-disposable plastic ware** must be treated before use to ensure that it is RNAse-free. Plastics should be thoroughly rinsed with 0.1 M NaOH, 1 mM EDTA followed by RNAse-free water. You can also use chloroform-resistant plastic ware rinsed with chloroform to inactivate RNAses.
- All solutions must be prepared with DEPC-treated RNAse-free water.
- Change gloves frequently and keep tubes closed.
- When handling RNA, reduce the preparation time as much as possible.
- Only use sterile disposable polypropylene tubes throughout the procedure.
- Always keep RNA samples on ice.

This kit should only be used by trained personnel.

Storage of RNA

Purified viral RNA can be stored at -80°C and is stable for several years at this condition.

Quantification

Quantification of DNA and RNA from this assay must be done by means of qPCR or Reverse Transcriptase qPCR. All other methods will be disturbed by the included Carrier RNA as well as DNA or RNA which is co-purified.

Ordering information

Product	Package Size	Catalogue No.
InviMag® Universal Kit /KF96	1 x 96 preparations	7450300100
InviMag® Universal Kit /KF96	5 x 96 preparations	7450300200
InviMag® Universal Kit /KF96/ w/o plastic	1 x 96 preparations	7450300150
InviMag® Universal Kit /KF96/ w/o plastic	5 x 96 preparations	7450300250

Related Products	Package Size	Catalogue No.
InviMag® Universal Kit / STARlet	4 x 96 preparations	7450330300
InviMag® Universal Kit / STARlet	24 x 96 preparations	7450330400
InviMag® Universal Kit/ IG	8 x 12 preps	2450120100
InviMag® Pathogen Kit /KF96	5 x 96 preparations	7450300200
InviMag® Pathogen Kit/ KFmL	75 preparations	2450110300
RTP® Pathogen Kit	250 preparations	1040500300
RTP® DNA/ RNA Virus Mini Kit	250 preparations	1040100300
RTP® Bacteria DNA Mini Kit	250 preparations	1033200300
InviMag® Virus RNA Mini Kit/ KF 96	5 x 96 preparations	7443300200
InviMag® Virus DNA Mini Kit/ KF 96	5 x 96 preparations	7442300200
InviMag® Virus DNA/ RNA Mini Kit/ KFmL	75 preparations	2441150200
InviMag® Virus DNA/ RNA Mini Kit	250 preparations	1440100300
InviMag® Bacteria DNA Mini Kit/ KFmL	75 preparations	2433150200

Possible suppliers for Isopropanol:

Carl Roth
2-Propanol
Rotipuran >99.7%, p.a., ACS, ISO
Order No. 6752

Applichem
2-Propanol
Order No. A3928

Sigma
2-Propanol
Order No. 59304-1L-F

Possible suppliers for centrifuges:

Eppendorf AG
22331 Hamburg, Germany
Phone: +49 (0) 40 53801 0
Fax: +49 (0) 40 53801 556
E-Mail: eppendorf@eppendorf.com
Internet: www.eppendorf.com

SIGMA Laborzentrifugen GmbH
37507 Osterode am Harz, Germany
Phone: +49-5522-5007-0
Fax: +49-5522-5007-12
E-Mail: info@sigma-zentrifugen.de
Internet: www.sigma-zentrifugen.de

KingFisher 96 and consumables

KingFisher 96, Magnetic Particle Processor,100-240V,50/60Hz	5400500
KingFisher 96 Head for Deep Well plate	24073430
KingFisher 96 tip comb for PCR magnets, 8 x 10 pcs / box	97002514
KingFisher 96 tip comb for KF magnets, 10 x 10 pcs / box	97002524
KingFisher 96 tip comb for DW magnets 10 x 10 pcs / box	97002534
KingFisher 96 KF plate (200ul) 48 plates / box	97002540
Microtiter deep well 96 plate, 50 plates/box	95040450

stratec molecular

STRATEC Molecular GmbH
Robert-Rössle-Str. 10
13125 Berlin, Germany

Phone: +49 30 94 89 29 01
Fax: +49 30 94 89 29 09
E-mail: info.berlin@stratec.com

www.stratec.com

1G7o02/05/2015